Navy Entomologists Assist CDC's Fight Against Malaria

by David Hoel, James Dunford, Daniel Szumlas, Craig Stoops and Robert Wirtz

Military entomologists serve in a variety of work positions – as deployed war fighters assisting preventive medicine units in theater, instructors, trainers, consultants, and researchers to name a few; and serve with many different organizations. Within the Navy, routine duty assignments include the Navy Entomology Center of Excellence (NECE) in Jacksonville, FL, the Navy and Marine Corps Public Health Center (the Navy's public health HQ) in Portsmouth, VA, three Navy Environmental and Preventive Medicine Units (Norfolk, San Diego, Pearl Harbor), and on Marine Corps Bases (Camp Lejeune, Camp Pendleton and Okinawa). We also serve in billets (work assignments) at the Armed Forces Pest Management Board in Silver Spring, MD as policy makers and with the Uniformed Services University of Health Sciences in Bethesda, MD as instructors. Less common assignments for Navy entomologists include the overseas research billets found in Cairo, Egypt at the US Navy Medical Research Unit No 3 (NAMRU-3), NAMRU-6 in Lima, Peru, and NAMRU-2 in Hawaii, soon to be relocated in Singapore. A liaison billet was created at the United States Department of Agriculture's Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE) to collaborate joint interest research projects for USDA and military entomology researchers. Several years ago an agreement was reached with Centers for Disease Control and Prevention (CDC) in Atlanta, GA to add two Navy entomology billets, one for a senior researcher, and a second training billet for junior or mid-grade officers. This narrative describes what Navy entomologists do at CDC and how our work here contributes to the overall mission of military entomology.

Figure 1: Larval collections from northern Uganda roadside ditch and borrow pit. Larvae are then reared to adults in an insectary and tested for insecticide resistance.

Report Documentation Page

Form Approved
OMB No. 0704-0188

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1. REPORT DATE **2013**	2. REPORT TYPE	3. DATES COVERED **00-00-2013 to 00-00-2013**

4. TITLE AND SUBTITLE **Navy Entomologists Assist CDC's Fight Against Malaria**	5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Medical Services Corps,Navy and Marine Corps Public Health Center Detachment,Centers for Disease Control and Prevention,Atlanta,GA,30329**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

The CDC grew out of an agency originally created to combat malaria in the United States, which had been a serious problem throughout the South since the founding of our country. As many as a million cases of malaria were occurring during most years of the 1930s and into the early 1940s, when the Office of Malaria Control in War Areas (MCWA), precursor to CDC, was created to combat the disease in the US. The inception of the MCWA could not have come at a better time as we were preparing for war in the malaria-rife Pacific, southern Europe, and North Africa. However, before we were to begin fighting malaria in faraway places, we needed to fight it here at home first, to ensure that the warfighters going overseas were not ill before hitting the beaches! In fact, some of the greatest threats of contracting malaria in the States occurred at basic training camps on southern military bases in such places as Fort Polk in the Louisiana swamps, Fort Jackson, SC, Camp Lejeune, NC, and Fort Benning, GA. An abundance of military bases throughout the South exposed many recruits to malarious mosquitoes before departing for their initial overseas assignments. It was also about this time when active duty entomologists played significant roles in preventing and reducing the number of malaria-related casualties for troops fighting in the South Pacific; towards the end of WWII there were nearly 200 entomologists assigned to over 150 epidemiology units.

Today, malaria is eliminated from the US, thanks in part to the many mosquito control organizations and control activities operating here, putting an end to endemic transmission by 1951 and controlling various outbreaks since then. However, military personnel still serve overseas in malarious areas such as Afghanistan and the Middle East, and are frequently tasked to serve in disaster relief and humanitarian missions in many places around the globe where malaria is present. The Department of Defense (DoD) created the Deployed Warfighter Protection Program (DWFP) to develop technologies, techniques, and products to protect deployed troops from vector-borne disease. Navy entomologists stationed at CDC work with CDC scientists on a variety of projects to fulfill the goals of DWFP and to assist with ongoing research of interest to CDC. They also serve as technical advisors to the President's Malaria Initiative (PMI), a US Government interagency initiative led by USAID and implemented with CDC, begun in 2005 to assist much of Africa and parts of Southeast Asia in their fight against malaria.

MALARIA AND PMI

Malaria is a sometimes fatal mosquito-borne parasitic disease that threatens roughly half the world's population, causing better than 200,000,000 clinical cases yearly in over 100 countries. Last year, malaria killed more than 600,000 people worldwide; most cases (70%) and the majority of deaths (90%) occurred in Africa, south of the Sahara Desert, where temperature, humidity and rainfall favor the production of large numbers of anopheline mosquitoes, the only vectors of the four *Plasmodium* parasites that infect and sicken humans (*P falciparum, P vivax, P ovale* and *P malariae*). Sadly, the majority of deaths occur in children less than five years of age. Until recently, malaria deaths had been much higher, closer to a million deaths a year, but thanks to global efforts to control malaria, death rates and illnesses are (at present) beginning to retreat. Threats to gains in malaria control from insecticide-resistant mosquitoes and bed net durability and insecticide longevity problems are just a few of many issues being examined by Navy entomologists supporting PMI.

Figure 2: Anopheline larvae collected from a roadside ditch in Uganda. Vegetation-free, sun-exposed ditches and borrow pits, used for making bricks, are favored larval habitat sites for *Anopheles gambiae*, an important malaria vector throughout much of Africa.

The President's Malaria Initiative was created by President Bush in 2005 as a five-year $1.2 billion program to reduce malaria deaths in African countries by 50%. Begun in 2006, PMI has grown from 3 countries to 19 sub-Saharan African countries and the Greater Mekong Sub-region in Asia. Thanks to a 5-year extension authorized under the Lantos-Hyde Act, the United States Government will continue to provide support through 2014. PMI relies on four proven interventions to reduce the burden of malaria: 1) the purchase and distribution of long-lasting insecticide treated bed nets (LLINs); 2) indoor residual spraying (IRS) of living quarters using long-lasting residual insecticides to control arthropophilic (human-feeding) anopheline malaria vectors; 3) intermittent preventive treatment for pregnant women (prevention using malaria drugs); and 4) case management including treatment of malaria cases with combinations of newer drugs called ACTs (artemisinin-based combination therapies) after confirmation of infection by microscopy or RDT (rapid diagnostic test).

Figure 3: At Camp Blanding in Florida, LT Ryan Larson of the Navy Entomology Center of Excellence sprays panels, made of materials typically found in African houses, with residual insecticide for longevity testing against *Anopheles gambiae* at CDC. CDC-DoD collaborations are mutually beneficial to both agencies.

PMI WORK

Senior Navy entomologists stationed at CDC serve as technical consultants to the PMI program. The vast majority of their work revolves around the IRS and LLIN interventions, and includes a wide variety of activities such as insecticide resistance monitoring, training host nationals on the World Health Organization (WHO) and CDC bottle bioassay techniques to detect insecticide resistance in malaria vectors, mosquito species identification, insecticide longevity testing with WHO cone assays on bed nets and IRS-treated walls, insectary startup consultation, help with or planning operational research programs in host countries, and mosquito surveillance with IRS partners; see Figures 1 and 2. We work primarily with Ministry of Health (MOH) and National Malaria Control Program (NMCP) personnel in host countries, but also with other in-country institutions, such as the Liberian Institute of Biomedical Research, the Uganda Virus Research Institute, and local universities involved with tropical disease training and research. We also interact with non-government organizations responsible for purchases and distribution of PMI commodities and other international players such as the Global Fund, World Bank, WHO and with PMI's IRS implementing partners such as Abt Associates and RTI International.

A year ago, the lead author was assigned as the entomology consultant to PMI teams in Uganda, Liberia, and Nigeria. Since that time, he has traveled to Uganda four times, and twice to Liberia and Nigeria. These three countries face tremendous challenges due to malaria. Everyone born and living in Liberia has had malaria at least once in their life and back-to-back civil wars decimated their public health infrastructure, compounding the problem of infection in recent years. In Uganda, nearly 40 percent of all outpatient visits to health clinics are due to malaria and over 90 percent of the country's 33 million residents live in highly malarious areas. Nigeria bears the brunt of the malaria burden in Africa, where it is estimated that a quarter of the world's malaria cases occur. Ghana, Mali and Malawi are other countries where Navy entomologists CDR Daniel Szumlas and LCDR Craig Stoops continue to have assigned PMI duties. Each country represents unique malaria scenarios due to topography, climate, anopheline species composition, mosquito feeding behaviors and changing insecticide resistance profiles.

Work has centered on developing the entomology portion of the annual PMI Malaria Operational

Plans for these countries and providing technical assistance and training to MOH employees. In Liberia, work has recently begun on developing a mobile insectary to enable the NMCP to colonize insecticide susceptible malaria mosquitoes for residual insecticide testing of IRS sprayed walls and long lasting insecticide-treated bed nets, which is essential in determining whether these insecticides are lasting as long as advertised. Shortfalls in product performance can greatly increase program costs. The insectary will be used to rear field-collected larvae to adults for local species determination and for training purposes. An insectary was recently completed in northern Uganda fulfilling these same needs and funded through the PMI program. We are in the process of purchasing mosquito surveillance equipment to help NMCPs establish or improve malaria surveillance programs for Nigeria, Liberia and Uganda. The goal is to provide enough equipment and training to the NMCP and MOH to enable them to eventually take over these operations and become self-sufficient in IRS and mosquito surveillance. The PMI effort in Liberia has been bolstered by technical assists, surveillance activities, and IRS technique training given to the Liberian NMCP and MOH by personnel from NECE and NAMRU-3. All of this work has occurred in conjunction with interagency country teams including epidemiologists and public health advisors to ensure the most effective use of entomology data to guide national programs.

COLLABORATIVE RESEARCH

In addition to PMI-related activities and overseas travel, Navy entomologists assigned to CDC are tasked with providing support to, as well as leading, several collaborative vector control research projects. The CDC bottle bioassay was developed by CDC entomologists as a quick, field expedient method of determining the insecticide susceptibility status of mosquitoes to a given insecticide or class of insecticides at a specific time and location. We are currently engaged with studies that aim to modify and enhance surveillance techniques to further develop this test. CDC senior research entomologist Dr William Brogdon, responsible for initial development of the CDC bottle bioassay, is currently leading an investigation to assess the frequency and intensity of insecticide resistance by tailoring assays to also allow for evaluation of the strength and resistance mechanism profile of resistance foci. This modified standard bioassay has been developed to determine insecticide resistance intensity in

mosquitoes by exposure to 2X, 5X, and 10X the diagnostic dose. Once resistance frequency and intensity are determined, discrimination between metabolic and target site resistance can also be determined using insecticide synergists in the standard bottle bioassay. Dr Brogdon's protocols are designed to more accurately reveal resistance strength and detoxification mechanisms in target mosquitoes. They allow for assessment of the maximum number of sites throughout a program area; thus, enabling intervention decision-making to be based on a more complete understanding of resistance at varying frequencies and intensities. Some of the laboratory work associated with these projects is being conducted by Navy entomologists at CDC.

Figure 4: CDR David Hoel collecting mosquitoes from a light trap placed under a "failed" bed net (with holes) to measure the degree of mosquito ingress into the bed net. This project is a collaboration between CDC and USDA–CMAVE, Gainesville, FL.

The CDC bottle bioassay is used primarily to determine susceptibility levels of mosquitoes to a variety of insecticides. However, CDR Daniel Szumlas and LCDR Toby Palmer, two Navy entomologists previously stationed at CDC, conducted bottle assays using house flies (*Musca domestica*) to determine diagnostic doses of insecticides needed for this group of public health pests. House flies represent a significant threat to deployed forces throughout the world because they aid in mechanically transmitting enteric pathogens that cause diarrheal diseases, a significant cause of morbidity to troops.

In the laboratory facilities at CDC's Roybal Campus in Atlanta, we are also using the CDC bottle bioassay to investigate the efficacy of candidate insecticidal compounds with the goal of determining the range of effective lethal concentrations for differing formulations of novel compounds. The goal is to find effective candidates with proven lethality to mosquitoes for future commercial development. C8910, a mixture of medium-chain octanoic, nonanoic and decanoic acids in equal parts, shows promising characteristics for use in public health vector control. At sufficient concentrations, this compound provides quick knockdown with no recovery. The fatty acids that comprise C8910 have been approved by the US FDA as food additives in the US since 1965 and are categorized as "Generally Recognized as Safe," We have conducted numerous tests that demonstrate its effectiveness against the malaria vector *Anopheles gambiae*, with plans to further evaluate this compound as well as similar ones against a variety of disease-carrying arthropods. Further development of C8910 and similar compounds may provide vector control specialists with effective, environmentally-safe insecticides for controlling insect disease vectors, as well as a sustainable alternative to a growing insecticide resistance crisis.

Camp Blanding Joint Training Center (Starke, FL), NECE and CDC teamed up in 2012 to conduct Indoor Residual Spray evaluations that intend to provide critical data on the residual longevity of WHO-approved (and those pending approval) insecticides used in IRS programs. NECE is well equipped to evaluate existing and new insecticides using state of the art equipment, and provide a tremendous resource to independently evaluate products already being implemented in malaria management programs. The geographical location, climate, and surrounding vegetation at Camp Blanding are ideal for reproducing 'real world' conditions that DoD and other vector control specialists encounter in the field. Wooden latrines, representing 'huts' that would be encountered

Figure 5: Broward College, FL interns Cristiane Gasparetto (left) and Rachel Cruz assisting with CDC bottle bioassays used to monitor insecticide resistance.

in sub-Saharan Africa, were positioned in open grassy areas adjacent to wooded habitat with moderate to dense understory. Each latrine was retrofitted interiorly with insecticide treated 12 by 12 inch panels made of various building materials (ie, sheet metal, plywood, cement, and mud/dung); see Figure 3. The panels were left exposed to environmental conditions and a subset of panels were collected and shipped to CDC at 1 week, 2 weeks, 1 month, 2 months, 4 months, and 6 months post-application. WHO cone bioassays were used against a susceptible strain of *An gambiae* available in colony at CDC. The primary objectives of this on-going study are to test a number of WHO-approved insecticides representing different insecticide classes, in order to determine the residual efficacy of each insecticide and whether including a pH buffer improves longevity. Ultimately, we will use the results to make recommendations to those already using or considering various insecticides for IRS programs.

A bed net study was also initiated in 2012 at Lower Suwannee National Wildlife Refuge, Levy County, FL with the assistance of personnel at USDA-CMAVE; see Figure 4. Because bed nets also represent one of the most effective malaria interventions used by global malaria programs including PMI, it is important to know when a bed net is considered no longer effective at preventing mosquito bites. Over time, bed nets often degrade by either losing their insecticide residual or (more concerning) by becoming frayed or replete with holes. In order to reveal the effects of hole size and obtain preliminary information towards determining bed net failure, a field experiment was conducted to appraise the ingress of host-seeking female mosquitoes into compromised un-treated bed nets. One hole each was centered and cut into each of four sides and the top of bed nets. Hole sizes were based on published WHO criteria for assessing bed net failure using a three hole size test criterion: finger (2.5 cm diameter), fist (10 cm diameter) and face (25 cm diameter) sized holes; the other two treatments consisted of a bed net with no holes and a site with no bed net (control). Field work in 2013 will include use of insecticide-treated bed nets, following the same design described above. Studies have only recently begun that address the physical and insecticidal longevity in bed nets used in government-funded antimalarial programs, so this information is needed to determine bed net field life.

Laboratory testing at the CDC insectary is also underway to determine the most effective combination of modified BG Sentinel traps and various attractants in capturing *An gambiae* mosquitoes, the primary malaria vector in Africa. Large room sized climate-controlled test chambers are used to conduct these tests. These test chambers were used by previous Navy entomologists in collaboration with the Mosquito and Fly Research Unit at CMAVE in Gainesville, FL to test various filth fly traps and devices that reduce fly populations and eliminate their associated nuisance and disease spreading potential. Anopheline mosquitoes often do not respond well to CDC light traps, and the DoD has an interest in developing better anopheline traps and attractants to enhance field surveillance of malaria mosquitoes to better protect troops deployed to malarious areas.

STUDENT INTERNS

The research aspect of our work at CDC has been greatly facilitated with the help of student interns brought in via a competitive internship program developed by LT James Dunford. An agreement between Broward College, Davie, FL and CDC has allowed us to bring in and mentor biology student interns to work alongside CDC and Navy entomologists to gain a better understanding of global public health as it relates to disease carrying arthropods; see Figure 5. Students have worked on several projects, including those described earlier. They also helped develop and appear in an instructional video on how to monitor insecticide resistance in the field. The work they conduct at CDC is counted as a three-credit course and included in their academic curriculum. As it turns out, both students chosen in 2012 had witnessed first-hand friends and close relatives afflicted by insect-borne disease in Brazil. As children, they had seen dengue outbreaks that closed schools and businesses in their towns and had family members chronically ill with Chagas disease. The internship provided both students a unique opportunity to learn about insects that transmit vector-borne diseases such as malaria, dengue and Chagas, diseases they were already familiar with. We can't say enough about how well the students performed, and how much we learned from them as we streamlined new protocols and procedures in the laboratory to evaluate various insect control methodologies. The program will continue in 2013.

CDC continues to be an excellent platform for us to promote Navy Medicine and Navy Entomology, and we will continue to use every opportunity to do so. We welcome visits from public health professionals and Navy entomologists across the fleet as well as from other branches and offer training on procedures we have learned while stationed here. CDC is a great location to work alongside some of the world's premier vector control specialists, epidemiologists, and humanitarians – and that is one of the main reasons Navy entomologists are here, to assist and learn from world class scientists and employ what we learn to better service deployed troops and countries in need of public health expertise. Service members are not immune to the various insect-borne diseases that occur worldwide and Navy entomologists play a major role in keeping members safe from these illnesses.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the US Government.

David Hoel
Commander
vap1@cdc.gov

James Dunford
Lieutenant
vit1@cdc.gov

Medical Service Corps
United States Navy
Navy and Marine Corps
Public Health Center
Detachment
Centers for Disease Control
and Prevention
Atlanta, GA 30329
404-718-4330

Daniel Szumlas
Commander
dszumlas@yahoo.com
Medical Service Corps
United States Navy
Walter Reed Military
Entomology Research
Program / Naval Medical
Research Center
Silver Spring, MD 20910
301-319-9646

Craig Stoops
Lieutenant Commander
Craig.Stoops@ars.usda.gov
Medical Service Corps
United States Navy
Center for Medical,
Agricultural and Veterinary
Entomology / United States
Department of Agriculture
Gainesville, FL 32608
352-374-5924

Robert Wirtz
Chief
rwirtz@cdc.gov
Entomology Branch
Department of Parasitic
Diseases and Malaria
Center for Global Health
Centers for Disease Control
and Prevention
Atlanta, GA 30329
404-718-4330